

Claims

- [c1] 1. A composition comprising a fluorophore compound, the fluorophore compound comprising a fluorophore group and a fluorescence quenching leaving group.
- [c2] 2. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least about 2 fold.
- [c3] 3. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least about 100 fold.
- [c4] 4. The composition of claim 1, wherein the fluorophore compound is characterized as having an efficiency of quenching of at least about 1000 fold.
- [c5] 5. The composition of claim 1, wherein the fluorophore compound is an organic compound, an organometallic compound, a nucleic acid, a peptide, a protein, a lipid, or a carbohydrate.
- [c6] 6. The composition of claim 1, wherein the fluorophore compound is a nucleic acid.

- [c7] 7. The composition of claim 6, wherein the nucleic acid is single stranded.
- [c8] 8. The composition of claim 6, wherein the nucleic acid is double stranded.
- [c9] 9. The composition of claim 6, wherein the quenching leaving group is attached to the 5' hydroxyl group of the nucleic acid.
- [c10] 10. The composition of claim 6, wherein the quenching leaving group is attached to a hydroxyl group other than the 5' hydroxyl group of the nucleic acid.
- [c11] 11. The composition of claim 6, wherein the fluorophore group is located 1, 2, or 3 nucleotides away from the quenching leaving group.
- [c12] 12. The composition of claim 1, wherein the fluorophore compound further comprises a nucleophilic group.
- [c13] 13. The composition of claim 12, wherein the nucleophilic group is a phosphorothioate or a phosphoroselenoate.
- [c14] 14. The composition of claim 1, wherein the fluorophore group is fluorescein, TAMRA, Cy3, Cy5, Cy5.5, BODIPY fluorophores, ROX, JOE, or Oregon Green.

- [c15] 15. The composition of claim 1, wherein the fluorescence quenching leaving group is a dabsyl group, a dimapdabsyl group, an azobenzene-sulfonyl group, an amino benzenesulfonyl group, a dialkylamino benzene-sulfonyl group, a nitro benzenesulfonyl group, a fluoro benzenesulfonyl group, a cyano benzenesulfonyl group, an arenesulfonyl group, an arylazo-substituted dabsyl group, or a gold particle conjugated to a sulfonyl leaving group.
- [c16] 16. The composition of claim 1, wherein the fluorophore compound is a peptide or a protein.
- [c17] 17. A method of detecting intramolecular chemical ligation, the method comprising:
providing a composition comprising a fluorophore compound, wherein the compound comprises a fluorophore group, a fluorescence quenching leaving group, and a nucleophilic group;
maintaining the composition under conditions suitable for intramolecular chemical ligation; and
determining the fluorescence of the composition.
- [c18] 18. The method of claim 17, further comprising determining the fluorescence of the composition before the maintaining step.

- [c19] 19. The method of claim 17, wherein the intramolecular chemical ligation occurs at a greater rate in the presence of an analyte than in the absence of an analyte.
- [c20] 20. The method of claim 17, wherein the determining step comprises visual detection, detection with a fluorescence microscope, detection with a fluorescence spectrometer, detection with a flow cytometer, or detection with a fluorescence microplate reader.
- [c21] 21. A method of detecting intermolecular chemical ligation, the method comprising:
providing a first composition comprising a fluorophore compound, wherein the fluorophore compound comprises a fluorophore group and a fluorescence quenching leaving group;
providing a second composition comprising a nucleophile compound, wherein the nucleophile compound comprises a nucleophilic group;
combining the first composition and the second composition to form a reaction mixture; and
determining the fluorescence of the reaction mixture.
- [c22] 22. The method of claim 21, further comprising determining the fluorescence of the first composition before the combining step.

- [c23] 23. The method of claim 21, wherein intramolecular chemical ligation occurs between the fluorophore compound and the nucleophile compound at a greater rate in the presence of an analyte than in the absence of an analyte.
- [c24] 24. The method of claim 21, wherein the determining step comprises visual detection, detection with a fluorescence microscope, detection with a fluorescence spectrometer, detection with a flow cytometer, or detection with a fluorescence microplate reader.
- [c25] 25. A method of detecting a nucleic acid sequence of interest, the method comprising:
providing a nucleic acid molecule suspected of comprising a nucleic sequence of interest;
providing a first nucleic acid probe that hybridizes to at least a portion of the nucleic acid sequence of interest;
providing a second nucleic acid probe that hybridizes to at least a portion of the nucleic acid sequence of interest adjacent to the first nucleic acid probe;
combining the nucleic acid molecule, the first nucleic acid probe, and the second nucleic acid probe to form a mixture;
maintaining the mixture under conditions suitable for hybridization of the first nucleic acid probe and the second nucleic acid probe to the nucleic acid molecule; and

determining the fluorescence of the mixture; wherein:
the first nucleic acid probe comprises a fluorophore group and a fluorescence quenching leaving group;
the second nucleic acid probe comprises a nucleophilic group; and
when the first nucleic acid probe and the second nucleic acid probe hybridize to the nucleic acid molecule, the nucleophilic group can displace the fluorescence quenching leaving group.

[c26] 26. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the 5' end of the first nucleic acid probe, and the nucleophilic group is covalently attached to the 3' end of the second nucleic acid probe.

[c27] 27. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the 3' end of the first nucleic acid probe, and the nucleophilic group is covalently attached to the 5' end of the second nucleic acid probe.

[c28] 28. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the 5' end of the first nucleic acid probe, and the nucleophilic group is covalently attached to the 3' end of the second nucleic acid probe.

- [c29] 29. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the first nucleic acid probe one nucleotide away from the fluorophore group.
- [c30] 30. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the first nucleic acid probe two nucleotides away from the fluorophore group.
- [c31] 31. The method of claim 25, wherein the fluorescence quenching leaving group is covalently attached to the first nucleic acid probe three nucleotides away from the fluorophore group.
- [c32] 32. The method of claim 25, wherein the nucleic acid molecule is DNA.
- [c33] 33. The method of claim 25, wherein the first nucleic acid probe is DNA.
- [c34] 34. The method of claim 25, wherein the second nucleic acid probe is DNA.
- [c35] 35. The method of claim 25, wherein the nucleic acid molecule is RNA, 2'-O-methyl-RNA, phosphorothioate DNA, locked nucleic acid ("LNA"), or PNA.

- [c36] 36. The method of claim 25, wherein the first nucleic acid probe is RNA, 2'-O-methyl-RNA, phosphorothioate DNA, locked nucleic acid ("LNA"), or PNA.
- [c37] 37. The method of claim 25, wherein the second nucleic acid probe is RNA, 2'-O-methyl-RNA, phosphorothioate DNA, locked nucleic acid ("LNA"), or PNA.
- [c38] 38. The method of claim 25, further comprising the step of determining the fluorescence of the mixture prior to the maintaining step.
- [c39] 39. The method of claim 25, wherein the determining step comprises visual detection, detection with a fluorescence microscope, detection with a fluorescence spectrometer, detection with a flow cytometer, or detection with a fluorescence microplate reader.
- [c40] 40. A kit for the detection of a nucleic acid sequence of interest, the kit comprising:
a first nucleic acid probe that hybridizes to at least a portion of the nucleic acid sequence of interest; and
a second nucleic acid probe that hybridizes to at least a portion of the nucleic acid sequence of interest adjacent to the first nucleic acid probe; wherein:
the first nucleic acid probe comprises fluorophore group and a fluorescence quenching leaving group;

the second nucleic acid probe comprises a nucleophilic group; and
when the first nucleic acid probe and the second nucleic acid probe hybridize to a nucleic acid molecule comprising the nucleic acid sequence of interest, the nucleophilic group can displace the fluorescence quenching leaving group.

[c41] 41. The kit of claim 40, wherein the fluorescence quenching leaving group is covalently attached to the 5' end of the first nucleic acid probe, and the nucleophilic group is covalently attached to the 3' end of the second nucleic acid probe.

[c42] 42. The kit of claim 40, wherein the fluorescence quenching leaving group is covalently attached to the 3' end of the first nucleic acid probe, and the nucleophilic group is covalently attached to the 5' end of the second nucleic acid probe.

[c43] 43. The kit of claim 40, wherein the fluorescence quenching leaving group is covalently attached to the first nucleic acid probe one nucleotide away from the fluorophore group.

[c44] 44. The kit of claim 40, wherein the fluorescence quenching leaving group is covalently attached to the

first nucleic acid probe two nucleotides away from the fluorophore group.

[c45] 45. The kit of claim 40, wherein the fluorescence quenching leaving group is covalently attached to the first nucleic acid probe three nucleotides away from the fluorophore group.

[c46] 46. The kit of claim 40, wherein the first nucleic acid probe is DNA.

[c47] 47. The kit of claim 40, wherein the second nucleic acid probe is DNA.

[c48] 48. The kit of claim 40, wherein the first nucleic acid probe is RNA, 2'-O-methyl-RNA, phosphorothioate DNA, locked nucleic acid ("LNA"), or PNA.

[c49] 49. The kit of claim 40, wherein the second nucleic acid probe is RNA, 2'-O-methyl-RNA, phosphorothioate DNA, locked nucleic acid ("LNA"), or PNA.